bio-protocol Preprint

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ATAC-Seq

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An abbreviated version of this protocol was published in eLIFE in Oct 2019

Herpes simplex viral nucleoprotein creates a competitive transcriptional environment facilitating robust viral transcription and host shut off

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Detailed protocol

DeLuca Lab ATAC-Sequencing Protocol **2020-04-28**

Adapted from Buenrostro et al., 2013, Nature Methods 10:1213–1218 and Buenrostro, et al., 2015, Curr. Protoc. Mol. Biol. 109:21.29.1-21.29.9.

Reagents:

- TDE1 Tagment DNA Enzyme (Illumina #15027865)
- TD Tagment DNA Buffer (Illumina #15027866)

Alternatively, a 2x tagmentation buffer (see receipe for 5X TmgAc-DMF Reaction Buffer in Grunenwald et al., 2010 patent 20100120098A1) can be prepared: 20 mM Tris(hydroxymethyl)aminomethane; 10 mM MgCl2; 20% (vol/vol) dimethylformamide. Before the addition of dimethylformamide, adjust the pH to 7.6 with 100% acetic acid.

- 10,000x Syber Green I (Live Technologies #S7563)
- NEBNext High-Fidelity 2x PCR Master Mix (NEB #M0541)
- Monarch PCR & DNA Cleanup Kit (NEB #TI030S)
- Qubit™ dsDNA HS Assay Kit (ThermoFisher #Q32851)
- Agilent DNA 7500 Kit (Agilent # 5067-1506)
- IDT Oligos

Oligo	Sequence (5'→3')	Barcode
Ad1	AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTG	
Ad2.1	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGT	TAAGGCGA
Ad2.2	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGT	CGTACTAG
Ad2.3	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGT	AGGCAGAA
Ad2.4	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGT	TCCTGAGC
Ad2.5	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGT	GGACTCCT
Ad2.6	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGT	TAGGCATG
Ad2.7	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGT	CTCTCTAC
Ad2.8	CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGGAGATGT	CAGAGAGG
Ad2.9	CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTCTCGTGGGCTCGGAGATGT	GCTACGCT
Ad2.10	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGGAGATGT	CGAGGCTG
Ad2.11	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGGAGATGT	AAGAGGCA
Ad2.12	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGGAGATGT	GTAGAGGA

Buffers:

**Autoclave solution to sterilize*

Tricine-buffered saline (TBS) (store at RT)

137 mM NaCl

5 mM KCl

0.5 mM MgCl

0.7 mM CaCl

25 mM Tricine

Adjust pH to 7.35 with 10 N NaOH

**Sterile filter solutions with 0.2 µm filter*

Lysis Buffer 1 (store at RT)

10mM Tris-HCl

10mM NaCl

3mM $MgCl_2$

Adjust pH to 7.4

Lysis Buffer 2 (prepare day of use and keep on ice)
10mM Tris-HCl
10mM NaCl
3mM $MgCl_2$
0.1% IGEPAL (N3500 Nonidet-P40 substitute CAS #9036-19-5)
Adjust pH to 7.4

Infection:

**In 60 mm tissue culture plates there are approximately 2 x 10^6 MRC5 cells at confluence. Make sure to plate and grow cells at 37^{o}C until they are completely confluent*

To infect at an MOI of 10 infect with 10^8 PFU/mL, 0.2mL/plate

- Dilute appropriate virus in cold TBS
- Aspirate media from plates
- Add 0.2 mL of viral inoculum
- Adsorb for 1 hour at room temperature (RT), rock gently every 10 minutes
- Aspirate viral inoculum
- Wash with 3 mL 37^{o}C TBS
- Add 3 mL 37^{o}C 2% FBS 1X DMEM media
- Incubate at 37^{o}C until time to prepare nuclei

Prepare Nuclei:

- Aspirate media from plates
- Wash cells with 3 mL chilled TBS
- Wash cells with 2 mL chilled Lysis Buffer 1. Aspirate wash completely.
- Add 1.5 mL Lysis Buffer 2. Use rubber scraper to dislodge all cells.
- Transfer cell suspension to 2 mL Dounce tissue grinder.
- Use "B" (tight) pestle for 5-10 strokes.

5 strokes was typically sufficient. Check for nuclei using trypan blue staining.

- Transfer aliquot of nuclear suspension to 1.5 mL epindorf tubes

This protocol is very dependent on cell number, so we usually try different aliquots of nuclei suspension in parallel to ensure optimal tagmentation. Generally we used aliquots ranging from 100-500 µL (1.3-6.7 x 10^5 cells if assuming ideal yield from nuclei isolation step).

- Spin 500 x g 10 min 4^{o}C
- Wash with 1 mL cold Lysis Buffer 1, do not dislodge pellet.
- Spin 500 x g 10 min 4^{o}C
- Carefully pipette off **all** supernatant, proceed immediately to tagmentation.

In our experience flash freezing nuclei severely impacted tagmentation efficiency, if possible proceed immediate with tagmentation.

Transposase and Purify:

- For nuclear pellets: Resuspend pellet in 25 µL 2x buffer TD and 5 µL TDE1. Bring total volume up to 50 µL with nuclease-free water.
- For CsCl-purified viral genome preps: Combine 50 ng viral DNA with 25 µL 2x buffer TD and 5 µL TDE1. Bring total volume up to 50 µL with nuclease-free water.
- Resuspend by pipetting up and down 10 times.
- Incubate 37^{o}C for 30 minutes.

Can increase tagmentation incubation to 1 hour if tagmentation is insufficient.

- Purify DNA using Monarch PCR & DNA Cleanup Kit
- Elute in 10 µL elution buffer. Store at -80^{o}C if necessary

PCR Amplify:

- Combine the following:

10 µL transposased DNA from prior step
10 µL nuclease free water
2.5 µL 25 µM PCR Primer (Ad1)
2.5 µL 25 µM Barcoded PCR Primer (Ad2.1-12)
25 µL NEBNext High-Fidelity 2x PCR Master Mix

- Amplify for 10 cycles at: 72^{o}C 5 min, 98^{o}C 30 sec, 10 cycles of [98^{o}C 10 sec, 63^{o}C 30 sec, 72^{o}C 1 min]

These cycling parameters were determined experimentally by doing a pilot qPCR containing:
1 µL transposased DNA
2 µL nuclease free water
1 µL 1:1100 diluted 10,000x Syber Green I
1 µL 6.25 µM PCR Primer (Ad1)
1 µL 6.25 µM Barcoded PCR Primer (Ad2.1-12)
5 µL NEBNext High-Fidelity 2x PCR Master Mix
Amplify for 20 cycles. 10 cycles was determine as optimal as it was the number of cycles required to reach approximately 1/3rd maximal intensity.

- Cleanup samples with Monarch PCR & DNA Cleanup Kit, elute with 10 µL elution buffer

Library QC and quantitation:

- Measure 1 µL sample with Qubit™ dsDNA HS Assay Kit. Use this to calculate an approximate nM concentration assuming average insert length of 100 bp. Samples were 50-80 nM.

- Measure 1 µL samples with Agilent DNA 7500 Kit. Use this to assess tagmentation efficiency.
- Combine samples at equimolar concentration based on Qubit calculation.
- We had our samples size selected for all fragments less than 250 bp using the Pippin Prep and sequenced on the NextSeq550 Mid-output 150 cycles to yield paired end data

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Dremel, S. and DeLuca, N. (2020). ATAC-Seq. Bio-protocol Preprint. bio-protocol.org/prep289.
2. Dremel, S. E. and DeLuca, N. A.(2019). Herpes simplex viral nucleoprotein creates a competitive transcriptional environment facilitating robust viral transcription and host shut off. eLIFE. DOI: 10.7554/eLife.51109